# WEST Search History

DATE: Monday, July 29, 2002

| Set Name side by side | Query | Hit Count | Set Name result set |
|---|---|---|---|
| *DB=USPT,PGPB; PLUR=YES; OP=ADJ* | | | |
| L7 | L6 and l4 | 22 | L7 |
| L6 | l5 and caspase and cleavage | 77 | L6 |
| L5 | fluorescent protein | 2468 | L5 |
| L4 | L1 or l2 or L3 | 9124 | L4 |
| L3 | (((530/350)!.CCLS.) ) | 7184 | L3 |
| L2 | (((435/219 )!.CCLS.) ) | 726 | L2 |
| L1 | ((435/183 )!.CCLS. ) | 1560 | L1 |

END OF SEARCH HISTORY

WEST

Generate Collection | Print

**Search Results** - Record(s) 1 through 10 of 22 returned.

1. Document ID: US 20020098550 A1

L7: Entry 1 of 22 File: PGPB Jul 25, 2002

PGPUB-DOCUMENT-NUMBER: 20020098550
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020098550 A1

TITLE: Death domain containing receptor 5

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

2. Document ID: US 20020090683 A1

L7: Entry 2 of 22 File: PGPB Jul 11, 2002

PGPUB-DOCUMENT-NUMBER: 20020090683
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020090683 A1

TITLE: TUMOR NECROSIS FACTOR-GAMMA

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

3. Document ID: US 20020081679 A1

L7: Entry 3 of 22 File: PGPB Jun 27, 2002

PGPUB-DOCUMENT-NUMBER: 20020081679
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020081679 A1

TITLE: NARC8 programmed cell-death-associated molecules and uses thereof

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

☐ 4. Document ID: US 20020076799 A1

L7: Entry 4 of 22 File: PGPB Jun 20, 2002

PGPUB-DOCUMENT-NUMBER: 20020076799
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020076799 A1

TITLE: Compositions and methods for modulating TGF-beta signaling

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

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☐ 5. Document ID: US 20020073441 A1

L7: Entry 5 of 22 File: PGPB Jun 13, 2002

PGPUB-DOCUMENT-NUMBER: 20020073441
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020073441 A1

TITLE: Compositions and methods for detecting proteolytic activity

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

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☐ 6. Document ID: US 20020072091 A1

L7: Entry 6 of 22 File: PGPB Jun 13, 2002

PGPUB-DOCUMENT-NUMBER: 20020072091
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020072091 A1

TITLE: Death domain containing receptor 5

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

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☐ 7. Document ID: US 20020064828 A1

L7: Entry 7 of 22 File: PGPB May 30, 2002

PGPUB-DOCUMENT-NUMBER: 20020064828
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020064828 A1

TITLE: Method of controlling the binding of calmyrin to presenilin

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

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☐ 8. Document ID: US 20020034784 A1

L7: Entry 8 of 22 File: PGPB Mar 21, 2002

PGPUB-DOCUMENT-NUMBER: 20020034784
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020034784 A1

TITLE: Novel molecules of the card-related protein family and uses thereof

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

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☐ 9. Document ID: US 20010029013 A1

L7: Entry 9 of 22 File: PGPB Oct 11, 2001

PGPUB-DOCUMENT-NUMBER: 20010029013
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20010029013 A1

TITLE: Bcl-G polypeptides, encoding nucleic acids and methods of use

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

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☐ 10. Document ID: US 6420534 B1

L7: Entry 10 of 22 File: USPT

US-PAT-NO: 6420534
DOCUMENT-IDENTIFIER: US 6420534 B1

TITLE: Alzheimer's disease secretase, APP substrates therefor, and uses thereof

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

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| Terms | Documents |
|---|---|
| L6 and l4 | 22 |

**Display Format:** - Change Format

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**Search Results** - Record(s) 11 through 20 of 22 returned.

☐ 11. Document ID: US 6417002 B1

L7: Entry 11 of 22 File: USPT

US-PAT-NO: 6417002
DOCUMENT-IDENTIFIER: US 6417002 B1

TITLE: Method for maintenance and selection of episomes

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

☐ 12. Document ID: US 6348573 B1

L7: Entry 12 of 22 File: USPT

US-PAT-NO: 6348573
DOCUMENT-IDENTIFIER: US 6348573 B1

TITLE: Compositions and methods for identifying apoptosis signaling pathway inhibitors and activators

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

☐ 13. Document ID: US 6342363 B1

L7: Entry 13 of 22 File: USPT

US-PAT-NO: 6342363
DOCUMENT-IDENTIFIER: US 6342363 B1

TITLE: Death domain containing receptor 4 nucleic acids and methods

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

☐ 14. Document ID: US 6333167 B1

L7: Entry 14 of 22 File: USPT

US-PAT-NO: 6333167
DOCUMENT-IDENTIFIER: US 6333167 B1

TITLE: Methods and reagents for identifying inhibitors of proteolysis of membrane-associated proteins

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

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☐ 15. Document ID: US 6306663 B1

L7: Entry 15 of 22 File: USPT

US-PAT-NO: 6306663
DOCUMENT-IDENTIFIER: US 6306663 B1

TITLE: Controlling protein levels in eucaryotic organisms

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

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☐ 16. Document ID: US 6277974 B1

L7: Entry 16 of 22 File: USPT

US-PAT-NO: 6277974
DOCUMENT-IDENTIFIER: US 6277974 B1

TITLE: Compositions and methods for diagnosing and treating conditions, disorders, or diseases involving cell death

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

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☐ 17. Document ID: US 6270964 B1

L7: Entry 17 of 22 File: USPT

US-PAT-NO: 6270964
DOCUMENT-IDENTIFIER: US 6270964 B1

TITLE: Protein fragment complementation assays for the detection of biological or drug interactions

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

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☐ 18. Document ID: US 6222017 B1

L7: Entry 18 of 22 File: USPT

US-PAT-NO: 6222017
DOCUMENT-IDENTIFIER: US 6222017 B1

TITLE: Mammalian pro-apoptotic Bok genes and their uses

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw. Desc | Image

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☐ 19. Document ID: US 6204035 B1

L7: Entry 19 of 22 File: USPT

US-PAT-NO: 6204035
DOCUMENT-IDENTIFIER: US 6204035 B1

TITLE: Methods and compositions to alter the cell surface expression of phosphatidylserine and other clot-promoting plasma membrane phospholipids

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw. Desc | Image

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☐ 20. Document ID: US 6177259 B1

L7: Entry 20 of 22 File: USPT

US-PAT-NO: 6177259
DOCUMENT-IDENTIFIER: US 6177259 B1

TITLE: Assays and kits for inhibition of polyglutamine-induced cell death

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw. Desc | Image

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Generate Collection | Print

| Terms | Documents |
|---|---|
| L6 and l4 | 22 |

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**Search Results** - Record(s) 21 through 22 of 22 returned.

☐ 21. Document ID: US 6159731 A

L7: Entry 21 of 22 File: USPT

US-PAT-NO: 6159731
DOCUMENT-IDENTIFIER: US 6159731 A

TITLE: Daxx, a Fas-binding protein that activates JNK and apoptosis

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw. Desc | Image

☐ 22. Document ID: US 6020143 A

L7: Entry 22 of 22 File: USPT

US-PAT-NO: 6020143
DOCUMENT-IDENTIFIER: US 6020143 A

TITLE: Method for identifying substances that affect the interaction of a presenilin-1-interacting protein with a mammalian presenilin-1 protein

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw. Desc | Image

Generate Collection | Print

| Terms | Documents |
|---|---|
| L6 and l4 | 22 |

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=> d his

     (FILE 'HOME' ENTERED AT 00:10:51 ON 29 JUL 2002)

     FILE 'HCAPLUS' ENTERED AT 00:10:57 ON 29 JUL 2002
L1         47626 S (PROTEIN OR POLYPEPTIDE) (L) FLUORES?
L2          1254 S L1 (L) (PROTEASE OR PROTEINASE) (L) FLUORES?
L3            45 S L2 (L) (LOOP OR SHEET)
L4            33 S L3 AND PD<19990418
L5             0 S L4 AND CASPASE
L6            41 S L2 (L) CASPASE
L7            18 S L6 AND PD<19990418
```

=> d ibib ab 1-18

L7 ANSWER 1 OF 18 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:474278 HCAPLUS
DOCUMENT NUMBER: 131:253874
TITLE: Stoichiometry, Free Energy, and Kinetic Aspects of Cytochrome c:Apaf-1 Binding in Apoptosis
AUTHOR(S): Purring, Cherie; Zou, Hua; Wang, Xiaodong; McLendon, George
CORPORATE SOURCE: Department of Chemistry, Princeton University, Princeton, NJ, 08544, USA
SOURCE: Journal of the American Chemical Society (**1999**), 121(32), 7435-7436
CODEN: JACSAT; ISSN: 0002-7863
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A few years ago, a discovery was made that cytochrome c, the paradigm for a small redox **protein**, also plays a major role in apoptosis. It has been shown that cytochrome c (Cc) along with ATP or dATP are necessary cofactors with apoptosis **protease** activating factor-1 (Apaf-1) for the activation of **caspase**-9. **Caspases**, a family of genetically conserved cysteine **proteases**, are key mediators of the execution phase of apoptosis. It has been indirectly inferred that cytochrome c forms a complex with Apaf-1, but until now the direct binding of cytochrome c has not been demonstrated. We have taken the first step in characterizing this mechanism by showing that cytochrome c does, in fact, bind strongly to Apaf-1, apparently with a 2:1 Cc:Apaf-1 stoichiometry. Using **fluorescence** polarization methods we have measured the binding const. for Apaf-1 and horse heart ZnCc, a **fluorescent** deriv. of Cc in which the heme iron is replaced by zinc. This species is known to be structurally equiv. to wild-type FeCc in binding to physiol. **protein** partners of Cc. We conclude from all the data that Apaf-1 binds two Cc mols. with high affinity (K > 1011 M-1). The observation of such strong binding has interesting implications for the cellular control of apoptosis. If the cytosolic vol. of a typical human cell is taken to be approx. 10-11 L, then the amt. of cytochrome c that must be released from the mitochondria for Apaf-1 binding to take place involves only individual mols. If so, then Cc:Apaf-1 binding is less well described as an equil. phenomenon, but rather as a cellular signaling event. In essence every Cc mol. released from the mitochondria could bind to Apaf-1 and presumably trigger **caspase**-9 activation.
REFERENCE COUNT: 14 THERE ARE 14 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 2 OF 18 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:447451 HCAPLUS
DOCUMENT NUMBER: 132:48398
TITLE: Role of acidic fibroblast growth factor in the mouse submandibular gland carcinogenesis
AUTHOR(S): Yoshioka, Yukio
CORPORATE SOURCE: Sch. Dent., Univ. Tokushima, Tokushima, 770-8504, Japan
SOURCE: Shikoku Shigakkai Zasshi (**1999**), 12(1), 133-156
CODEN: SSZAED; ISSN: 0914-6091
PUBLISHER: Shikoku Shigakkai
DOCUMENT TYPE: Journal
LANGUAGE: Japanese
AB The effects of acidic fibroblast growth factor (aFGF) on the growth of mouse submandibular gland carcinoma cells and 9,10-dimethyl-1,2-benzanthracene (DMBA)-induced mouse submandibular gland carcinogenesis were examd. Recombinant human aFGF, bovine brain aFGF (bo-aFGF), recombinant human FGF-4 and 5 all stimulated the growth of mouse submandibular gland carcinoma YTcl 1-2 cells which were derived from an undifferentiated carcinoma. Goal: To det. whether aFGF could modify the

DMBA-induced mouse submandibular gland carcinogenesis. Groups 1 and 2 female mice received 1 mg of DMBA into their left submandibular glands. Thereafter, they were given a s.c. administration of either 0.5 mg/50 .mu.L bo-aFGP or vehicle, resp., three times a week for 10 wk. Groups 3 and 4 mice were given either bo-aFGF or vehicle only. Sixteen weeks after the beginning of the expt. the incidence of carcinomas in Group 1 was significantly ($p < 0.05$) greater than that in Group 2. The mean wt. of submandibular glands in Group 1 mice was the greatest indicating that bo-aFGF can promote the development of submandibular gland carcinoma. AFGF was demonstrated in granular convoluted tubule cells and ductal cells in the normal submandibular glands and carcinomas by immunohistochem. staining. Immunoblot anal. detected **protein** with mol. wt. of 18 kDa in normal and tumor tissues and YTcl 1-2 cells. Expression of aFGF mRNA was also demonstrated in all exptl. samples and YTcl 1-2 cells. Immunohistochem. study and immunoblot anal. revealed the expression of FGF receptor (FGFR)-1 and -4, but not FGFR-2 and -3 in normal submandibular gland and tumor tissues, indicating that aFGF binds to these receptors of the submandibular gland carcinomas and may stimulate cell growth by autocrine and paracrine mechanisms. The growth signal of aFGF should be transmitted into cells by tyrosine phosphorylation of its receptor. Tyrphostins are artificial and low mol. wt. compds. that specifically inhibit **protein** tyrosine kinases. Tyrphostins 9, 23, 47, B42, and B46 suppressed the growth of YTcl 1-2 cells, and tyrphostin 9 was found to he the most potent inhibitor; the IC50 was estd. to be 0.1 .mu.M. Furthermore, the growth of nude mouse tumors induced by YTcl 1-2 cell inoculation was suppressed by intra-tumor administration of tyrphostin 9 ($P < 0.001$), indicating that it was effective in vitro and in vivo Tyrphostin 9 inhibited tyrosine-phosphorylation of membrane **proteins** including FGFR-1 in the aFGF-treated YTcl 1-2 cells Flow cytometric anal. of the tyrphostin 9-treated cell DNA indicated that tyrphostin 9 induced the arrest of cell cycle and apoptosis. Indeed, DNA fragmentation of the treated YTcl 1-2 cells was demonstrated by **fluorescent** DNA staining with Hochest 33258 and terminal deoxynucleotidyl transferase mediated dUTP-biotin nick end labeling method. Electron microscopic study also revealed nuclear disorganization, development of cytoplasmic vacuoles, and decrease of mitochondria in the tyrphostin-treated cells. The dysfunction of mitochondria membrane was also indicated by the leakage of incorporated Rhodamin 123 from mitochondria. Dose- and time-dependent activation of apoptosis-assocd. **protease, caspase**-3, was detected after the treatment with this tyrphostin. These results suggest that aFGF plays a role in the development of mouse submandibular gland carcinomas and the growth of carcinoma cells, and that tyrosine kinase inhibitor, tyrphostin 9, can be used as an inhibitor for the submandibular gland carcinoma.

L7 ANSWER 3 OF 18 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:397367 HCAPLUS
DOCUMENT NUMBER: 131:181483
TITLE: Novel mutant green **fluorescent protein protease** substrates reveal the activation of specific **caspases** during apoptosis
AUTHOR(S): Mahajan, Nupam P.; Harrison-Shostak, D. Corinne; Michaux, Jennifer; Herman, Brian
CORPORATE SOURCE: The Lineberger Comprehensive Cancer Center and Department of Cell Biology and Anatomy, University of North Carolina at Chapel Hill, Chapel Hill, NC, 27599, USA
SOURCE: Chemistry & Biology (**1999**), 6(6), 401-409
CODEN: CBOLE2; ISSN: 1074-5521
PUBLISHER: Current Biology Publications
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The caspase-mediated proteolysis of many cellular proteins is a crit. event during programmed cell death or apoptosis. It is important to det. which caspases are activated in mammalian cells, and where and when activation occurs, upon receipt of specific death stimuli. Such

information would be useful in the design of strategies to regulate the activation of caspases during apoptosis. We developed two novel fluorescent substrates that were specifically cleaved by caspase-1 or caspase-3. For in vitro studies, four-amino-acid recognition sequences, YVAD for caspase-1 and DEVD for caspase-3, were introduced between blue fluorescent protein (BFP) and green fluorescent protein (GFP), expressed in bacteria and purified. For in vivo studies, YVAD and DEVD were introduced between cyan fluorescent protein and yellow fluorescent protein, and expression was monitored in live mammalian cells. The proximity between fluorophores was detd. using fluorescence resonance energy transfer. Purified substrates were cleaved following exposure to purified caspase-1 and caspase-3. In Cos-7 cells, caspase-1 and caspase-3 substrates were cleaved upon induction of apoptosis with staurosporine, a protein-kinase inhibitor, whereas caspase-3 but not caspase-1 substrate was cleaved upon treatment of cells with the DNA-damaging agent mitomycin c. These substrates allow the spatial activation of specific members of the caspase family to be deciphered during the initiation and execution phase of programmed cell death, and allow activation of specific caspases to be monitored both in vivo and in vitro. This technol. is also likely to be useful for high-throughput screening of reagents that modulate caspase activity.

REFERENCE COUNT: 18 THERE ARE 18 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 4 OF 18 HCAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1999:378030 HCAPLUS

DOCUMENT NUMBER: 131:179425

TITLE: Inhibition of caspase activity does not prevent the signaling phase of apoptosis in prostate cancer cells

AUTHOR(S): Denmeade, Samuel R.; Lin, Xiaohui S.; Tombal, Bertrand; Isaacs, John T.

CORPORATE SOURCE: Johns Hopkins Oncology Center, Johns Hopkins University School of Medicine, Baltimore, MD, USA

SOURCE: Prostate (New York) (**1999**), 39(4), 269-279
CODEN: PRSTDS; ISSN: 0270-4137

PUBLISHER: Wiley-Liss, Inc.

DOCUMENT TYPE: Journal

LANGUAGE: English

AB BACKGROUND. **Caspases** are a family of cysteine **proteases** capable of characteristically cleaving after an aspartic acid residue. Various members of the **caspase** family (e.g., **caspases** 8 and 9) have been implicated as crit. initiators in the signaling phase, while others (e.g., **caspases** 3, 6,and 7) have been implicated in the effector or execution phase of apoptosis. Thapsigargin (TG) is capable of inducing cell proliferation-independent apoptosis of prostate cancer cells. This study was undertaken to det. if **caspase** inhibition can prevent TG- or 5-fluorodeoxyuridine (5-FrdU)-induced apoptosis in prostate cancer cells. METHODS. **Caspase** activity was evaluated by Western blot anal. of the cleavage of retinoblastoma (Rb) **protein**, a **caspase** substrate during TG-induced death of prostate cancer cells. In addn., hydrolysis of **caspase**-specific **fluorescent** peptide substrates was assayed in lysates from TG-treated cells. Clonogenic survival assays were performed following treatment of rat AT3 and human TSU-Pr1 prostate cancer cell lines with TG and 5-FrdU in the presence and absence of peptide **caspase** inhibitors. AT3.1 cells transfected with the crmA gene, encoding a viral **protein** with **caspase**-inhibitory activity, were also tested for clonogenic survival following TG and 5-FrdU exposure. RESULTS. During treatment with TG, Rb is first dephosphorylated and then proteolytically cleaved into 100-kDa and 40-kDa forms, indicative of **caspase** activity. A 6-8-fold increase in class II (i.e., **caspases** 3, 7, and 10) hydrolysis of the **caspase** substrate Z-DEVD-AFC was obsd. after 24 h of TG or 5-FrdU. AT3 cells expressing crmA (i.e., an inhibitor of **caspases** 1, 4, and 8) were not protected from apoptosis induced by TG or 5-FrdU. The **caspase** inhibitors Z-DEVD-fmk (i.e., an inhibitor of **caspases** 3, 7, and 10) and Z-VAD-fmk (i.e., a general

**caspase** inhibitor) were also unable to protect TSU and AT3 cells from apoptosis induced by TG or 5-FrdU. CONCLUSIONS. **Caspase** activation may play a role in the downstream effector phase of the apoptotic cascade; however, in this study, **caspase** inhibition did not prevent the signaling phase of apoptosis induced by two agents with distinct mechanisms of cytotoxicity, TG or 5-FrdU. These results suggest that **caspase** inhibition by recently described endogenous **caspase** inhibitors should not lead to development of resistance to TG. A strategy for targeting TG's unique cytotoxicity to metastatic prostate cancer cells is currently under development.

REFERENCE COUNT: 58 THERE ARE 58 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 5 OF 18 HCAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1999:336264 HCAPLUS

DOCUMENT NUMBER: 131:100472

TITLE: Localization, regulation and possible consequences of apoptotic protease-activating factor-1 (Apaf-1) expression in granulosa cells of the mouse ovary

AUTHOR(S): Robles, Rodolfo; Tao, Xiao-Jing; Trbovich, Alexander M.; Maravei, Daniel V.; Nahum, Ravit; Perez, Gloria I.; Tilly, Kim I.; Tilly, Jonathan L.

CORPORATE SOURCE: Vincent Center for Reproductive Biology, Department of Obstetrics and Gynecology, Massachusetts General Hospital/Harvard Medical School, Boston, MA, 02114-2696, USA

SOURCE: Endocrinology (**1999**), 140(6), 2641-2644
CODEN: ENDOAO; ISSN: 0013-7227

PUBLISHER: Endocrine Society

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The recent characterization of apoptotic **protease**-activating factor-1 (Apaf-1) in vertebrates as a putative homolog of the Caenorhabditis elegans gene, ced-4, indicates that the third major arm of the C. elegans programmed cell death machinery has also been conserved through evolution. Although apoptosis is now known to be important for ovarian follicular atresia in vertebrates, nothing is known of the role of Apaf-1 in ovarian function. Herein the authors show by immunohistochem. anal. that Apaf-1 is abundant in granulosa cells of early antral follicles, whereas in vivo gonadotropin priming completely suppresses Apaf-1 expression and granulosa cell apoptosis. Western blot anal. of fractionated **protein** exts. prepd. from granulosa cells before and after in vitro culture without hormonal support to induce apoptosis indicated that mitochondrial cytochrome c release, a biochem. step required for the activation of Apaf-1, occurs in granulosa cells cultured in vitro. Moreover, Western blot anal. of procaspase-3 processing, a principal downstream event set in motion by activated Apaf-1, indicated that healthy granulosa cells possess almost exclusively the inactive (pro-) form of the enzyme, whereas granulosa cells deprived of hormonal support rapidly process procaspase-3 to the active enzyme. Lastly, the authors show that serum-starved granulosa cells activate **caspase** -3-like enzymes both prior to and after nuclear pyknosis, as revealed by a single-cell **fluorescent caspase** activity assay. These data, combined with previous observations regarding the role of homologs of the two other C. elegans cell death regulatory genes, ced-9 (Bcl-2 family members) and ced-3 (**caspases**), in atresia fully support the hypothesis that granulosa cell apoptosis is precisely coordinated by all three major arms of a cell death program conserved through evolution.

REFERENCE COUNT: 18 THERE ARE 18 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 6 OF 18 HCAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1999:309578 HCAPLUS

DOCUMENT NUMBER: 131:100642

TITLE: Caspase-mediated cleavage of cytoskeletal actin plays a positive role in the process of morphological apoptosis

AUTHOR(S): Mashima, Tetsuo; Naito, Mikihiko; Tsuruo, Takashi
CORPORATE SOURCE: Laboratory of Biomedical Research, Institute of Molecular and Cellular Biosciences, University of Tokyo, Tokyo, 113, Japan
SOURCE: Oncogene (**1999**), 18(15), 2423-2430
CODEN: ONCNES; ISSN: 0950-9232
PUBLISHER: Stockton Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Tumors result from the imbalance between cell growth and apoptosis. One of the characteristic changes in cancers is the abnormality in cytoskeleton, which suggests some roles of cytoskeletal **proteins** in tumorigenesis or the maintenance of tumor cells. Previously the authors showed that cytoskeletal actin is the substrate of **caspases**, the **proteases** responsible for apoptosis, while the role of actin cleavage in apoptosis remained unknown. To examine the cleavage of actin in vivo, the authors extensively performed immunoblot anal. using actin fragment-specific antibody. Here, the authors showed that, in some solid tumor cells, induction of apoptosis was accompanied by **caspase**-dependent actin-cleavage to 15 and 31 kDa fragments in vivo. To elucidate the role of actin-cleavage further, the authors introduced actin cleaved-fragments. The authors found that ectopic expression of an actin 15 kDa fragment induces morphol. changes resembling those of apoptotic cells. The expression of the actin fragment induced a dramatic change of cellular actin localization, as visualized by enhanced green **fluorescent protein** (EGFP)-tagged actin, while the actin fragment expression did not cause **caspase** activation nor the cleavage of a marker substrate **protein**, poly (ADP-ribose) polymerase. These results indicate that actin cleavage could play a pos. role in the morphol. changes of apoptosis downstream of **caspase** activation.

REFERENCE COUNT: 46 THERE ARE 46 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 7 OF 18 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:242174 HCAPLUS
DOCUMENT NUMBER: 131:72217
TITLE: Expression of extended polyglutamine sequentially activates initiator and effector caspases
AUTHOR(S): Miyashita, Toshiyuki; Matsui, Jun; Ohtsuka, Yuko; U, Mami; Fujishima, Sayaka; Okamura-Oho, Yuko; Inoue, Tadashi; Yamada, Masao
CORPORATE SOURCE: Department of Genetics, National Children's Medical Research Center, Tokyo, 154-8509, Japan
SOURCE: Biochemical and Biophysical Research Communications ( **1999**), 257(3), 724-730
CODEN: BBRCA9; ISSN: 0006-291X
PUBLISHER: Academic Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB To date, eight neurodegenerative disorders, including Huntington's disease and dentatorubral-pallidoluysian atrophy, have been identified to be caused by expansion of a CAG repeat coding for a polyglutamine (polyQ) stretch. It is, however, unclear how polyQ expansion mediates neuronal cell death obsd. in these disorders. Here, the authors have established a tetracycline-regulated expression system producing 19 and 56 repeats of glutamine fused with green **fluorescent protein**. Induced expression of the 56 polyQ stretch, but not of the 19 polyQ stretch, caused marked nuclear aggregation and apoptotic morphol. changes of the nucleus. In vitro enzyme assays and Western blotting showed that polyQ56 expression sequentially activated initiator and effector **caspases**, such as **caspase**-8 or -9, and **caspase** -3, resp. Furthermore, using cell-permeable fluorogenic substrate, the activation of **caspase**-3-like **proteases** was demonstrated in intact cells with aggregated polyQ. This is the first direct evidence that the expression of extended polyQ activates **caspases** and together with the previous findings that some of the

products of genes responsible for CAG repeat diseases are substrates of **caspase**-3 indicates an important role of **caspases** in the pathogenesis of these diseases. (c) 1999 Academic Press.
REFERENCE COUNT: 28 THERE ARE 28 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 8 OF 18 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:37963 HCAPLUS
DOCUMENT NUMBER: 130:164184
TITLE: Toxic bile salts induce rodent hepatocyte apoptosis via direct activation of Fas
AUTHOR(S): Faubion, William A.; Guicciardi, M. Eugenia; Miyoshi, Hideyuki; Bronk, Steven F.; Roberts, Patricia J.; Svingen, Phyllis A.; Kaufmann, Scott H.; Gores, Gregory J.
CORPORATE SOURCE: Division of Gastroenterology and Hepatology, Mayo Medical School, Clinic, and Foundation, Rochester, MN, 55905, USA
SOURCE: Journal of Clinical Investigation (**1999**), 103(1), 137-145
CODEN: JCINAO; ISSN: 0021-9738
PUBLISHER: American Society for Clinical Investigation
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Cholestatic liver injury appears to result from the induction of hepatocyte apoptosis by toxic bile salts such as glycochenodeoxycholate (GCDC). Previous studies from this lab. indicate that cathepsin B is a downstream effector **protease** during the hepatocyte apoptotic process. Because **caspases** can initiate apoptosis, the present studies were undertaken to det. the role of **caspases** in cathepsin B activation. Immunoblotting of GCDC-treated McNtcp.24 hepatoma cells demonstrated cleavage of poly(ADP-ribose) polymerase and lamin B1 to fragments that indicate activation of effector **caspases**. Transfection with CrmA, an inhibitor of **caspase** 8, prevented GCDC-induced cathepsin B activation and apoptosis. Consistent with these results, an increase in **caspase** 8-like activity was obsd. in GCDC-treated cells. Examn. of the mechanism of GCDC-induced **caspase** 8 activation revealed that dominant-neg. FADD inhibited apoptosis and that hepatocytes isolated from Fas-deficient lymphoproliferative mice were resistant to GCDC-induced apoptosis. After GCDC treatment, immunopptn. expts. demonstrated Fas oligomerization, and confocal microscopy demonstrated .DELTA.FADD-GFP (Fas-assocd. death domain-green **fluorescent protein**), aggregation in the absence of detectable Fas ligand mRNA. Collectively, these data suggest that GCDC-induced hepatocyte apoptosis involves ligand-independent oligomerization of Fas, recruitment of FADD, activation of **caspase** 8, and subsequent activation of effector **proteases**, including downstream **caspases** and cathepsin B.
REFERENCE COUNT: 42 THERE ARE 42 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 9 OF 18 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1998:797528 HCAPLUS
DOCUMENT NUMBER: 130:135767
TITLE: TRAIL/Apo2L activates c-Jun NH2-terminal kinase (JNK) via caspase-dependent and caspase-independent pathways
AUTHOR(S): Muhlenbeck, Frank; Haas, Elvira; Schwenzer, Ralph; Schubert, Gisela; Grell, Matthias; Smith, Craig; Scheurich, Peter; Wajant, Harald
CORPORATE SOURCE: Institute of Cell Biology and Immunology, University of Stuttgart, Stuttgart, 70569, Germany
SOURCE: Journal of Biological Chemistry (**1998**), 273(49), 33091-33098
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal

LANGUAGE: English

AB In this study, we show that TRAIL (tumor necrosis factor-related apoptosis-inducing ligand), also called Apo2L, activates the c-Jun N-terminal kinase (JNK). Interestingly, TRAIL-induced JNK activation occurs in a cell type-specific manner. In HeLa cells, TRAIL-induced JNK activation can be completely blocked with the cysteine **protease** inhibitor zVAD-fmk, whereas the same inhibitor has no, or even a stimulatory, effect on JNK activation in Kym-1 cells. Hence, TRAIL can engage at least two independent pathways leading to JNK activation, one that is cysteine **protease**-dependent and one that is cysteine **protease**-independent. To investigate whether the cysteine **protease**-dependent signaling of TRAIL leading to JNK activation is related to the apoptotic pathway engaged by this ligand, we investigated HeLa cells stably overexpressing a dominant neg. mutant of FADD (Fas-assocg. **protein** with death domain) (GFP(green **fluorescent protein**).DELTA.FADD). In these cells, TRAIL-induced cell death and activation of the apoptosis executioner **caspase**-8 (FLICE/MACH) and **caspase**-3 (YAMA, CPP-32, Apopain), that belong to **caspase** subfamily of cysteine **proteases**, were abrogated, whereas JNK activation remained unaffected and was still sensitive toward z-VAD-fmk. Similar data were found in HeLa cells overexpressing Apo1/Fas and GFP.DELTA.FADD upon stimulation with agonistic antibodies. These data suggest that crosslinking of the TRAIL receptors and Apo1/Fas, resp., engages a FADD-dependent pathway leading to the activation of apoptotic **caspases** and, in parallel, a FADD-independent pathway leading to the stimulation of one or more cysteine **proteases** capable to activate JNK but not sufficient for the induction of cell death.

REFERENCE COUNT: 69 THERE ARE 69 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 10 OF 18 HCAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1998:766905 HCAPLUS

DOCUMENT NUMBER: 130:137744

TITLE: Hypoxia induces apoptosis in human neuroblastoma SK-N-MC cells by caspase activation accompanying cytochrome c release from mitochondria

AUTHOR(S): Araya, Runa; Uehara, Takashi; Nomura, Yasuyuki

CORPORATE SOURCE: Graduate School of Pharmaceutical Sciences, Department of Pharmacology, Hokkaido University, Sapporo, 060-0812, Japan

SOURCE: FEBS Letters (**1998**), 439(1,2), 168-172
CODEN: FEBLAL; ISSN: 0014-5793

PUBLISHER: Elsevier Science B.V.

DOCUMENT TYPE: Journal

LANGUAGE: English

AB We have attempted to elucidate the mechanism of apoptotic cell death induced by hypoxia (very low oxygen conditions) in neuronal cells. Human neuroblastoma SK-N-MC cells under hypoxic conditions resulted in apoptosis in a time-dependent manner estd. by DNA fragmentation assay and nuclear morphol. stained with **fluorescent** chromatin dye. Pretreatment with Z-Asp-CH2-DCB, a **caspase** inhibitor, suppressed the DNA ladder in response to hypoxia in a concn.-dependent manner. An increase in **caspase**-3-like **protease** (DEVDase) activity was obsd. during apoptosis, but no **caspase**-1 activity (YVADase) was detected. To confirm the involvement of **caspase**-3 during apoptosis, Western blot anal. was performed using anti-**caspase**-3 antibody. The 20- and 17-kDa **proteins**, corresponding to the active products of **caspase**-3, were generated in hypoxia-challenged lysates in which processing of the full length form of **caspase**-3 was evident. With a time course similar to this **caspase**-3 activation, hypoxic stress caused the cleavage of PARP, yielding an 85-kDa fragment typical of **caspase** activity. In addn., **caspase**-2 was also activated by hypoxia, and the stress elicited the release of cytochrome c into the cytosol during apoptosis. These results suggest that **caspase** activation and cytochrome c release play roles in hypoxia-induced neuronal apoptosis.

REFERENCE COUNT: 34 THERE ARE 34 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

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L7 ANSWER 11 OF 18 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1998:716682 HCAPLUS
DOCUMENT NUMBER: 130:104925
TITLE: Role of Caspases in Immunotoxin-Induced Apoptosis of Cancer Cells
AUTHOR(S): Keppler-Hafkemeyer, Andrea; Brinkmann, Ulrich; Pastan, Ira
CORPORATE SOURCE: Laboratory of Molecular Biology Division of Basic Sciences National Cancer Institute, National Institutes of Health, Bethesda, MD, 20892, USA
SOURCE: Biochemistry (**1998**), 37(48), 16934-16942
CODEN: BICHAW; ISSN: 0006-2960
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Immunotoxins composed of antibodies linked to plant or bacterial toxins are being evaluated in the treatment of cancer. It is known that the toxin moieties of immunotoxins, including Pseudomonas exotoxin A (PE), diphtheria toxin, and ricin, are capable of inducing apoptosis. Since the efficiency of induction of apoptosis and the apoptosis pathway may have direct effects on the therapeutic usefulness of immunotoxins, we have studied how B3(Fv)-PE38, a genetically engineered immunotoxin in which the Fv fragment of an antibody is fused to a mutated form of PE, induces apoptosis of the MCF-7 breast cancer cell line. We show for the first time that a PE-contg. immunotoxin activates ICE/ced-3 **proteases**, now termed **caspases**, and causes characteristic cleavage of the "death substrate" poly(ADP)-ribose polymerase (PARP) to an 89 kDa fragment with a time course of cleavage comparable to that induced by TNF.alpha.. Also the **fluorescent** substrate, DEVD-AFC, is cleaved 2-4-fold more rapidly by lysates from B3(Fv)-PE38 treated MCF-7 cells than untreated control cells, suggesting that a CPP32-like **caspase** is involved in B3(Fv)-PE38-mediated apoptosis. B3(Fv)-PE38-induced PARP cleavage is inhibited by several **protease** inhibitors known to inhibit **caspases** (zVAD-fmk, zDEVD-fmk, zIETD-fmk) as well as by overexpression of Bcl-2 providing addnl. evidence for **caspase** involvement. ZVAD-fmk, a broad spectrum inhibitor of most mammalian **caspases**, prevents the early morphol. changes and loss of cell membrane integrity produced by B3(Fv)-PE38, but not its ability to inhibit **protein** synthesis, arrest cell growth, and subsequently kill cells. Despite inhibition of apoptosis, the immunotoxin is still capable of selective cell killing, which indicates that B3(Fv)-PE38 kills cells by two mechanisms: one requires **caspase** activation, and the other is due to the arrest of **protein** synthesis caused by inactivation of elongation factor 2. The fact that an immunotoxin can specifically kill tumor cells without the need of inducing apoptosis makes such agents esp. valuable for the treatment of cancers that are protected against apoptosis, e.g., by overexpression of Bcl-2.
REFERENCE COUNT: 51 THERE ARE 51 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 12 OF 18 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1998:638936 HCAPLUS
DOCUMENT NUMBER: 129:341895
TITLE: Prodomain-dependent nuclear localization of the caspase-2 (Nedd2) precursor. A novel function for a caspase prodomain
AUTHOR(S): Colussi, Paul A.; Harvey, Natasha L.; Kumar, Sharad
CORPORATE SOURCE: Hanson Centre for Cancer Research, Institute of Medical and Veterinary Science, Adelaide, 5000, Australia
SOURCE: Journal of Biological Chemistry (**1998**), 273(38), 24535-24542
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular

Biology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB **Caspases** are cysteine **proteases** that play an essential role in apoptosis by cleaving several key cellular **proteins**. Despite their function in apoptosis, little is known about where in the cell they are localized and whether they are translocated to specific cellular compartments upon activation. In the present paper, using Aequorea victoria green **fluorescent protein** (GFP) fusion constructs, the authors detd. the localization of Nedd2 (mouse **caspase**-2) and showed that both precursor and processed **caspase**-2 localize to the cytoplasmic and the nuclear compartments of transfected NIH-3T3 cells. The authors demonstrated that the nuclear localization of **caspase**-2 was strictly dependent on the presence of the pro-domain. A **caspase**-2 pro-domain-GFP localized to dot- and fiber-like structures, mostly in the nucleus, whereas a **protein** lacking the pro-domain was largely concd. in the cytoplasm. It was also shown that an N-terminal fusion of the pro-domain of **caspase**-2 to **caspase**-3 mediated nuclear transport of **caspase**-3, which is normally localized in the cytoplasm. These results suggest that, in addn. to roles in dimerization and recruitment through adaptors, the **caspase**-2 pro-domain has a novel function in nuclear transport.

L7 ANSWER 13 OF 18 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1998:541778 HCAPLUS
DOCUMENT NUMBER: 129:243599
TITLE: Intracellular aggregate formation of dentatorubral-pallidoluysian atrophy (DRPLA) protein with the extended polyglutamine
AUTHOR(S): Miyashita, Toshiyuki; Nagao, Kazuaki; Ohmi, Kazuhiro; Yanagisawa, Hiroko; Okamura-Oho, Yuko; Yamada, Masao
CORPORATE SOURCE: Department of Genetics and Pathology, National Children's Medical Research Center, Tokyo, 154-8509, Japan
SOURCE: Biochemical and Biophysical Research Communications ( **1998**), 249(1), 96-102
CODEN: BBRCA9; ISSN: 0006-291X
PUBLISHER: Academic Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Dentatorubral-pallidoluysian atrophy (DRPLA) is an autosomal dominant neurodegenerative disorder caused by the abnormal CAG triplet-repeat expansion resulting in an elongated polyglutamine (polyQ) stretch. We have recently showed that the DRPLA **protein** is cleaved during apoptosis by **caspase**-3, one of the cysteine **protease** family members known to be activated during apoptosis. We report here the subcellular localization of the DRPLA **protein** by fusing the green **fluorescent protein** as a tag. The full length DRPLA **protein** is localized predominantly but not exclusively in the nucleus regardless of the length of the polyQ stretch. In contrast, an N-terminal-deleted fragment contg. polyQ produced by the proteolytic cleavage with **caspase**-3 is found both in the nucleus and the cytoplasm. Moreover, the same fragment with the elongated polyQ showed aggregation when overexpressed. Some cells with aggregate formation showed apoptotic phenotype. These findings raise the possibility that the DRPLA **protein** processed by **caspase**-3 may lead to aggregation of the **protein** resulting in the development of neurodegeneration. (c) 1998 Academic Press.

L7 ANSWER 14 OF 18 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1998:412009 HCAPLUS
DOCUMENT NUMBER: 129:202056
TITLE: Perforin-dependent nuclear entry of granzyme B precedes apoptosis, and is not a consequence of nuclear membrane dysfunction
AUTHOR(S): Trapani, Joseph A.; Jans, Patricia; Smyth, Mark J.;

AUTHOR(S): Froelich, Christopher J.; Williams, Elizabeth A.; Sutton, Vivien R.; Jans, David A.
CORPORATE SOURCE: Cellular Cytotoxicity Laboratory, The Austin Research Institute, Heidelberg, 3084, Australia
SOURCE: Cell Death and Differentiation (**1998**), 5(6), 488-496
CODEN: CDDIEK; ISSN: 1350-9047
PUBLISHER: Stockton Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Killer lymphocytes utilize the synergy of a membranolytic **protein**, perforin, and the serine **protease** granzyme B (grB) to induce target cell apoptosis, however the mechanism of this synergy remains incompletely defined. The authors have previously shown that perforin specifically induces the redistribution of cytoplasmic grB into the nucleus of dying cells, however a causal role for nuclear targeting of grB in cell death has not been demonstrated. In the present study, the authors used confocal laser scanning microscopy (CLSM) to det. whether the nuclear accumulation of **fluoresceinated** (FITC-) grB precedes or is a consequence of apoptosis. Two distinct and mutually exclusive cellular responses were obsd. in FDC-P1 cells: (i) up to 50% of the cells rapidly accumulated FITC-grB in the nucleus (maximal at 7 min; t1/2 of 2 min) and underwent apoptosis; (ii) the remaining cells took up FITC-grB only into the cytoplasm, and escaped apoptosis. Under these conditions, DNA fragmentation was not obsd. for at least 13 min, indicating nuclear accumulation of grB preceded the execution phase of apoptosis. Furthermore, nuclear import of grB proceeded through an intact nuclear membrane, as the nuclei of cells whose cytoplasm was pre-loaded with 70 kDa FITC-dextran excluded dextran for up to 90 min while still undergoing apoptosis in response to perforin and grB. These findings indicated that perforin-induced nuclear accumulation of grB precedes apoptosis, and is not a byproduct of **caspase**-induced nuclear membrane degrdn. The cell membrane lesions formed by perforin in these expts. were not large enough to permit a 13 kDa **protein** (yeast cdk p13suc) access into the cytoplasm, but an 8 kDa **protein** (bacterial azurin) was able to equilibrate between the cytosol and the exterior. Therefore, transmembrane pores large enough to allow passive diffusion of grB (32 kDa) into the cell are not necessary for apoptosis. Rather, a perforin-dependent signal results in a redistribution of grB from the cytoplasm to the nucleus, where it may contribute to the nuclear changes assocd. with apoptosis.

L7 ANSWER 15 OF 18 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1998:300008 HCAPLUS
DOCUMENT NUMBER: 129:90001
TITLE: Inhibition of epidermal growth factor receptor kinase induces protease-dependent apoptosis in human colon cancer cells
AUTHOR(S): Karnes, William E., Jr.; Weller, Shaun G.; Adjei, Philip N.; Kottke, Timothy J.; Glenn, Kahlil S.; Gores, Gregory J.; Kaufmann, Scott H.
CORPORATE SOURCE: Division of Gastroenterology, Mayo Clinic, Rochester, MN, USA
SOURCE: Gastroenterology (**1998**), 114(5), 930-939
CODEN: GASTAB; ISSN: 0016-5085
PUBLISHER: W. B. Saunders Co.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The epidermal growth factor receptor (EGFR) is under investigation as a therapeutic target for cancers. Colon cancer cell lines are variably dependent on autocrine stimulation of EGFR. We therefore examd. the effects of a selective EGFR tyrosine kinase inhibitor, PD 153035, on proliferation and survival of five colon cancer cell lines whose autonomous proliferation is either EGFR ligand dependent or EGFR ligand independent. Effects of inhibitors were screened by MTS growth assays, [3H]thymidine incorporation, terminal deoxynucleotidyl transferase-medaited deoxyuridine triphosphate nick-end labeling assay,

**fluorescence** microscopy, immunoblotting, and in vitro **protease** assays. PD 153035 caused dose-dependent cytostasis (200 nmol/L to 1 .mu.mol/L) and apoptosis (>10 .mu.mol/L) in ligand-dependent cell lines and caused variable apoptosis (>10 .mu.mol/L) but no cytostasis in ligand-independent cell lines. Apoptosis induced by 10 .mu.mol/L PD 153035 was not assocd. with induction of p53 **protein** expression but was accompanied by activation of **caspases** that cleave poly(ADP-ribose) polymerase, lamin B1, and Bcl-2. Inhibition of **caspase** 3-like **protease** activity by DEVD-fluoromethylketone significantly delayed the onset of PD 153035-induced apoptosis. The EGFR tyrosine kinase inhibitor PD 153035 induces cytostasis and **caspase**-dependent apoptosis in EGFR ligand-dependent colon cancer cell lines. These observations encourage further investigation of EGFR tyrosine kinase inhibitors for treatment of colorectal neoplasms.

L7 ANSWER 16 OF 18 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1998:272306 HCAPLUS
DOCUMENT NUMBER: 129:25240
TITLE: Detection of programmed cell death using fluorescence energy transfer
AUTHOR(S): Xu, Xiang; Gerard, Amy L. V.; Huang, Betty C. B.; Anderson, David C.; Payan, Donald G.; Luo, Ying
CORPORATE SOURCE: Rigel, Inc., Sunnyvale, CA, 94086, USA
SOURCE: Nucleic Acids Research (**1998**), 26(8), 2034-2035
CODEN: NARHAD; ISSN: 0305-1048
PUBLISHER: Oxford University Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB **Fluorescence** energy transfer (FRET) can be generated when green **fluorescent protein** (GFP) and blue **fluorescent protein** (BFP) are covalently linked together by a short peptide. Cleavage of this linkage by **protease** completely eliminates FRET effect. **Caspase**-3 (CPP32) is an important cellular **protease** activated during programmed cell death. An 18 amino acid peptide contg. CPP32 recognition sequence, DEVD, was used to link GFP and BFP together. CPP32 activation can be monitored by FRET assay during the apoptosis process.

L7 ANSWER 17 OF 18 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1998:94205 HCAPLUS
DOCUMENT NUMBER: 128:226014
TITLE: Prevention of hypoxic liver cell necrosis by in vivo human bcl-2 gene transfection
AUTHOR(S): Yamabe, Kazuo; Shimizu, Shigeomi; Kamiike, Wataru; Waguri, Satoshi; Eguchi, Yutaka; Hasegawa, Jun-Ichi; Okuno, Shin-Ichiro; Yoshioka, Yasuhiko; Ito, Toshinori; Sawa, Yoshiki; Uchiyama, Yasuo; Tsujimoto, Yoshihide; Matsuda, Hikaru
CORPORATE SOURCE: The First Department of Surgery, Biomedical Research Center, Osaka University Medical School, Suita, 565, Japan
SOURCE: Biochemical and Biophysical Research Communications ( **1998**), 243(1), 217-223
CODEN: BBRCA9; ISSN: 0006-291X
PUBLISHER: Academic Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Prevention of hypoxic cell death is a key to successful liver transplantation. The authors developed a new method for preventing liver hypoxic cell death by introducing an anti-cell death gene directly into rat livers. When the human bcl-2 gene (hbcl-2) was directly transfected into rat livers together with nonhistone chromosomal **protein** high-mobility group 1 (HMG1) by the hemagglutinating virus of Japan (Sendai virus; HVJ) -liposome method, human Bcl-2 **protein** (hBcl-2) was efficiently expressed. Electron microscopy and

**fluorescence** microscopy revealed that hepatocytes expressing exogenous hBcl-2 were almost completely protected from hypoxic cell necrosis. The expression of the hBcl-2 also inhibited activation of **caspase**-3 (-like) **proteases** and liver dysfunction. Thus, transfection of the hbcl-2 gene through HVJ-liposome method is useful to prevent liver cell necrosis induced by hypoxia. This finding could lead to new strategies to avoid the hypoxic cell death, the major problem in liver transplantation.

L7 ANSWER 18 OF 18 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1998:52938 HCAPLUS
DOCUMENT NUMBER: 128:152960
TITLE: Dominant-negative FADD inhibits TNFR60-, Fas/Apo1- and TRAIL-R/Apo2-mediated cell death but not gene induction
AUTHOR(S): Wajant, Harald; Johannes, Franz-Josef; Haas, Elvira; Siemienski, Katrin; Schwenzer, Ralph; Schubert, Gisela; Weiss, Tilo; Grell, Matthias; Scheurich, Peter
CORPORATE SOURCE: Institute of Cell Biology and Immunology, University of Stuttgart, Stuttgart, 70569, Germany
SOURCE: Current Biology (**1998**), 8(2), 113-116
CODEN: CUBLE2; ISSN: 0960-9822
PUBLISHER: Current Biology Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Fas/Apo1 and other cytotoxic receptors of the tumor necrosis factor receptor (TNFR) family contain a cytoplasmic death domain (DD) that activates the apoptotic process by interacting with the DD-contg. adaptor **proteins** TNFR-assocd. DD **protein** (TRADD) and Fas-assocd. DD **protein** (FADD/MORT1), leading to the activation of cysteine **proteases** of the **caspase** family. Stimulation of Fas/Apo1 leads to the formation of a receptor-bound death-inducing signaling complex (DISC), consisting of FADD and two different forms of **caspase**-8. Transient expression of a dominant-neg. mutant of FADD impairs TNFR60-mediated and Fas/Apo1-mediated apoptosis, but has no effect on TNF-related apoptosis-inducing ligand (TRAIL/Apo2L)-induced cell death. To study the function of FADD in DD-receptor signaling in more detail, we established HeLa cells that stably expressed a green **fluorescent protein** (GFP)-tagged dominant-neg. mutant of FADD, GFP-.DELTA.FADD. Interestingly, expression of this mutant inhibited cell death induced by TNFR60, Fas/Apo1 and TRAIL-R/Apo2. In addn., GFP-.DELTA.FADD did not interfere with TNF-mediated gene induction or with activation of NF-.kappa.B or Jun N-terminal kinase (JNK), demonstrating that FADD is part of the TNFR60-initiated apoptotic pathway but does not play a role in TNFR60-mediated gene induction. Fas/Apo1-mediated activation of JNK was unaffected by the expression of GFP-.DELTA.FADD, suggesting that in Fas/Apo1 signaling the apoptotic pathway and the activation of JNK diverge at a level proximal to the receptor, upstream of or parallel to FADD.